POSTER SESSION 1
2:00 - 3:00 PM

CHARACTERIZING IMMUNE RESPONSES TO AAV9 IN A MOUSE MODEL OF DUCHENNE MUSCULAR DYSTROPHY

MICHAEL EMAMI (MBIDP/ CDB, Spencer lab)

Duchenne muscular dystrophy (DMD) is an X-linked disease caused by out-of-frame mutations in the DMD gene. Gene replacement strategies use AAV to deliver a truncated DMD gene, and are currently in phase I/II trials; however, serious adverse events (SAEs) have been reported in some patients who received AAV-microdystrophin. This response was unforeseen, as trial participants are screened for neutralizing antibody (NAb) titers against the AAV capsid and for pre-existing T cell responses against dystrophin. It is unclear why certain patients develop SAEs and the specific immune responses that arise following AAV gene therapy. In order to comprehensively and unbiasedly characterize AAV-induced immune responses in vivo, we dosed a dystrophic mouse model with AAV serotype 9 carrying a vector encoding CRISPR/Cas9 and mCherry reporter and performed 10x Genomics single cell RNA-sequencing (scRNA-seq). We injected AAV9-mCherry and PBMCs were isolated and analyzed prior to AAV administration and then 2 weeks post-AAV administration. After a single injection of AAV-mCherry, phenotypic shifts in monocytes, NK cells, B cells, and T cells were observed. We also tested the effect of two AAV-CRISPR mCherry injections. Upon second exposure to AAV-mCherry, mCherry expression was not detected, suggesting that immunological rejection had occurred. Concomitant with rejection was a more profound shift in immune clusters by scRNA-seq (compared to the single injection cohort), and an emergence of new immune cell phenotypes. Additional analysis and validation studies are needed to identify critical immune cell populations and genes that elicit responses to AAV. These studies will enable the identification of new target genes involved in immune responses to AAV.

EXTENDED BETA-SHEETS CONTRIBUTE TO REVERSIBLE AMYLOID FORMATION

DECLAN EVANS (MBIDP/ BBSB, Houk lab)

The assembly of proteins into fibrillar amyloid structures has been traditionally considered to be pathologic and irreversible. Recent evidence suggests amyloid structures that form reversibly, derived from protein low-complexity domains, may play a role in functional cell processes. Atomic-level structures of amyloid proteins known to undergo irreversible and/or reversible aggregation have been determined, and analysis of these structures highlights key elements in fibril morphology that may influence reversibility. While all amyloid fibrils are primarily composed of repeating layers of beta-sheets, fibril structures of proteins known to reversibly aggregate have an enrichment of highly extended non-ideal beta-sheets. Quantum mechanical calculations of pleated- and extended-beta sheet amyloid structures show that extended backbones decrease the energy required to separate strand pairs. Non-covalent interaction analysis shows that the extended beta-sheets may be stabilized by intra-residue interactions between the amide proton and carbonyl oxygen, known as C5 hydrogen-bonding. These findings identify a key structural element that may regulate reversible amyloid assembly.

A MECHANISTIC STUDY OF THE REGULATION OF STING BY CHOLESTEROL

IAN FORD (MBIDP/ IMMP, Bensinger lab)

Recent investigations have shown surprising links between STING (STimulator of INterferon Genes) signaling and lipid metabolism. Previous studies from our lab and others have demonstrated that disruption of cholesterol biosynthetic programs in macrophages results in heightened STING signaling. Importantly, replenishing cholesterol normalizes STING signaling within these cells. However, the molecular mechanism underlying the regulation of STING by lipids has not been clear. In this study, we first show that addition of exogenous cholesterol inhibits STING translocation from the endoplasmic reticulum to perinuclear puncta even in the presence of its activating ligand, thus limiting the ability of STING to induce TBK1-IRF3 signaling and Type I IFN expression. Conversely, genetic or pharmacological depletion of cellular cholesterol results in spontaneous STING activation and translocation. Using a cholesterol-mimetic probe, trans-sterol, we demonstrate that cholesterol binds directly to STING, and in silico analysis of STING reveals two putative cholesterol binding motifs, termed CRAC and CARC. We show that disruptions in the CARC and/or CRAC domains within STING renders its activity insensitive to changes in cellular cholesterol and eliminates binding to trans-sterol, supporting a direct binding mechanism for regulation of STING by cholesterol. The results of this study demonstrate a complex crosstalk between immune signaling and cholesterol metabolism in the context of acute infection.

CHARACTERIZING HUMAN SKELETAL MUSCLE EXTRACELLULAR MATRIX AND INVESTIGATING ITS ROLE IN MODULATING SATELLITE CELL FATE ACROSS HUMAN DEVELOPMENT

DEVIN GIBBS (MBIDP/ CDB, Pyle & Crosbie labs)

Endogenous skeletal muscle stem cells (MuSC), known as satellite cells (SC) in adults and skeletal muscle progenitor cells (SMPC) during fetal development, are the leading players in skeletal muscle development and its adaptive response to injury. SMPCs derived from human induced pluripotent stem cells (hiPSC) are transcriptionally immature and inefficiently differentiate to form myotubes when engrafted in vivo. The maturation of hiPSC-SMPCs into SCs is required in order to develop an hiPSC based therapy for skeletal muscle diseases such as the most prevalent lethal genetic disease in children, Duchenne Muscular Dystrophy (DMD). Stem cells interact with their surrounding extracellular matrix (ECM) during tissue development and regeneration, with research demonstrating that matrix-ligand interactions have the ability to dictate cell differentiation and cell fate. We hypothesize that the crucial maturation factor for SMPC transition to SC resides within the developing extracellular matrix. We have developed an on-slide and in-culture dish decellularization protocol for both wildtype and DMD murine skeletal muscle as well as adult and developing human skeletal muscle to generate acellular ECM myoscaffolds on which cells can be cultured and seeded upon to assess SC, SMPC, and hiPSC-SMPC ECM interactions. The cells exhibited robust motility, adhesion, and differentiation on healthy myoscaffolds. While Fibrotic myoscaffolds from DMD muscle lead to decreased cell fusion and proliferation and increased cell death. Interestingly, when cells were cultured on myoscaffolds generated from developing human muscle the rate of SMPC proliferation was increased and upon manual induction of differentiation, cells maintained MuSC identity longer than in any other culture condition.

SINGLE CELL SEQUENCING OF HUMAN WHITE ADIPOSE TISSUE IDENTIFIES NOVEL CELL STATES IN HEALTH AND IN OBESITY

ANDREW HILDRETH (MBIDP/ IMMP, O'Sullivan lab)

White adipose tissue (WAT) is an essential regulator of energy storage and systemic metabolic homeostasis. Regulatory networks consisting of immune and structural cells are necessary to maintain WAT homeostasis, which can become impaired during obesity in mammals. Using single-cell transcriptomics validated by flow cytometry, we unveil the cellular landscape of the stromal vascular fraction (SVF) of healthy lean and obese human WAT. We report novel subsets, activation states, developmental trajectories and upstream regulators of adipose innate lymphoid cells (ILCs), dendritic cells (DCs) and macrophages that accumulate in healthy obese individuals. Analysis of cell-cell ligand receptor interactions reveals a switch from immunoregulatory pathways in lean WAT to structural cell, DC, ILC and macrophage mediated inflammatory networks in obese WAT. These results provide a detailed cellular atlas of homeostatic and inflammatory networks in healthy human WAT.

BIOACTIVE PRODIGININE SCAFFOLDS & AN UNUSUAL PHOTOREARRANGEMENT

EVAN HURLOW (Chem & Biochem, Harran lab)

Prodiginines are tripyrrole natural products possessing a wide variety of biological properties. Our lab's recent total synthesis of anti-cancer marineosin A utilized an unusual photorearrangement of an ansa-bridged pyridine macrocycle. We have explored the mechanism of this reaction using GC/MS time series and DFT calculations, and have elucidated pathways leading to the formation of unique heteromacrocycle products. It was recently discovered that the prodiginine compound butylcycloheptyl prodigiosin (bPGN) is capable of binding to and inhibiting the processing of pre-microRNA-21, a precursor to the oncogenic mircoRNA-21. In collaboration with Prof. Feng Guo, we plan to obtain an x-ray diffraction structure of bPGN interacting with pre-microRNA-21 and use this to optimize the compound's properties.

CHEMOPROTEOMICS AS A TOOL TO PROBE RNA-BINDING PROTEINS

ASHLEY JULIO (Chem & Biochem/ BMSB, Backus lab)

Chemoproteomics is a powerful tool that enables the high-throughput analysis of protein function and targetability. Through this approach, cysteine-reactive compounds have been shown to target RNA-binding proteins (RBPs), demonstrating that they are amenable to modification by drug-like molecules, despite being previously considered "undruggable." Based on this exciting discovery, our lab is working to develop new chemical probes and chemoproteomic platforms to explore aspects of RBP structure, function, and druggability. In the project presented here, we assess the potential utility of halogenated nucleoside analogs as fully functionalized metabolic probes. We show that both 5-iodouridine and 5-iodocytidine can be metabolically incorporated into cellular RNA and detected by conjugation to biotin via palladium-catalyzed Suzuki-Miyaura cross-coupling. In this vein, certain halogenated nucleosides may be selectively incorporated into cellular RNA with heterologous expression of RNA biosynthesis enzymes, such as UPRT, UCK2, and UMPS. Such selective incorporation will enable RNA labeling and RBP profiling in a cell-type specific manner. Furthermore, our preliminary studies suggest that iodinated nucleoside analogs can function as reagents for interactome studies, as irradiation of labeled RNA initiates transfer of the iodine species to nearby tyrosine and histidine residues. This method will enable the site-specific identification of RNA binding sites among a multitude of RBPs through Suzuki-Miyaura biotinylation and proteomic analysis. Collectively, our data suggest that halogenated nucleosides will be effective chemical probes for various aspects of RNA biology. We anticipate that the development of this strategy will prove useful for applications in profiling gene expression, RBP dynamics, and drug target sites.

HETEROBIFUNCTIONAL BENZALDEHYDE-MALEIMIDE LINKER FOR N-TERMINAL PROTEIN CONJUGATION

GRACE KUNKEL (Chem & Biochem, Maynard lab)

Due to the various reactive functionalities present on peptides and proteins, rationally designed conjugation linkers are necessary in order to achieve site-specificity. In this work, a benzaldehyde linker is synthesized, which undergoes reductive amination in the presence of sodium cyanoborohydride, to selectively conjugate at the N-terminus. The N-terminus is an attractive site for widely applicable conjugation because it facilitates a single conjugation for most peptides and proteins, which each have only one N-terminus. The N-terminus is also distanced from most binding sites. Furthermore, benzaldehyde mediated linkage preserves the positive charge on the N-terminal amine, which can allow for greater stability. Bridged with the addition of a hydrolysis-resistant alkyl chain, the synthesized linker herein also contains a maleimide, which reacts specifically with thiols through a Michael-addition. This handle allows conjugation access to any thiol-containing small molecule or polymer target. A suite of polymers, such as p(NIPAM), p(PEGMA), and trehalose, has been synthesized by reversible addition chain transfer (RAFT) controlled polymerization. The trithiocarbonate end-group afforded by the chain transfer agent is readily reduced to a thiol in the presence of amines. Using the resultant free thiols, this polymer library was conjugated

to GCSF as a proof-of-concept model protein. Therefore, a wide range of polymers can be conjugated to proteins with specificity at the N-terminal using the linker of this study.

FLUOROUS SOLUBLE CYANINE DYES FOR VISUALIZING PERFLUOROCARBONS IN LIVING SYSTEMS

IRENE LIM (Chem & Biochem, Sletten lab)

The bioorthogonal nature of perfluorocarbons provides a unique platform for introducing dynamic nano- and microdroplets into cells and organisms. To monitor the localization and deformation of the droplets, fluorous soluble fluorophores that are compatible with standard fluorescent protein markers and applicable to biology are necessary. Here, we introduce fluorous cyanine dyes. We evaluate the changes in photophysical properties imparted by the fluorous phase. We showcase the utility of the fluorous soluble pentamethine cyanine dye for tracking the localization of perfluorocarbon nanoemulsions in macrophage cells and for measurements of mechanical forces in multicellular spheroids and zebrafish embryonic tissues. These studies demonstrate that the red-shifted cyanine dyes offer spectral flexibility in multiplexed imaging experiments and enhanced precision in force measurements.

ELUCIDATING ALPHAVIRAL DETERMINANTS OF SENSITIVITY TO ZINC FINGER ANTIVIRAL PROTEIN (ZAP)

LEANN NGUYEN (MBIDP/ IMMP, Li lab)

The type I interferon (IFN) response defends against viral infection by stimulating expression of antiviral genes known as IFN-stimulated genes (ISGs). One such ISG is zinc finger antiviral protein (ZAP), which inhibits a wide spectrum of RNA and DNA viruses. ZAP recognizes viral CG dinucleotide motifs, recruits mRNA degradation machineries, and inhibits viral mRNA translation. However, the mechanism by which ZAP recognizes a broad yet specific range of viruses remains elusive. We study ZAP by investigating its translational inhibition of alphaviruses, which exhibit a diversity of sensitivity to ZAP inhibition: Sindbis virus (SINV) and Ross River virus are more sensitive to ZAP inhibition than o'nyong'nyong virus (ONNV) and chikungunya virus. Although ZAP is a CG dinucleotide sensor, the frequency of CG elements across these viral genomes does not correlate with ZAP inhibition, suggesting additional viral determinants of sensitivity. We hypothesize that resistant alphaviruses have modified or eliminated sequence determinants to escape detection by ZAP or encode viral antagonist(s) of ZAP. Identifying viral determinants will elucidate the mechanism by which ZAP targets many but not all viruses. By generating chimeras between SINV (ZAP-sensitive) and ONNV (ZAP-resistant) viruses, we determined that the non-structural gene region contains the ZAP sensitivity determinants. We are further characterizing these determinants by generating additional chimeric alphaviruses, overexpressing non-structural proteins, and assaying ZAP binding to viral RNAs. Our work will reveal novel viral strategies for evading detection by the type I IFN system and potential ways in which ZAP can be harnessed for therapeutic purposes.

AAV-MEDIATED GENE THERAPY EXTENDS LIFESPAN AND IMPROVES UREAGENESIS IN A MOUSE MODEL OF CPS1 DEFICIENCY

MATT NITZAHN (MBIDP/ CDB, Lipshutz lab)

The mammalian urea cycle is responsible for the removal of toxic waste nitrogen originating from protein catabolism. Carbamoyl phosphate synthetase 1 (CPS1) deficiency is a rare autosomal recessive disorder resulting from the loss of the first enzyme of this cycle, CPS1, and causes life-threatening hyperammonemia and cerebral edema. Current treatment strategies rely principally on dietary protein restriction and ammonia scavenger drugs, which are only modestly effective at best and unable to prevent recurrent crises and neurocognitive decline. While liver transplantation is curative (but does not reverse the accumulative neurological insults), limited access to healthy donor organs and complications from long-term immunosuppression restrict its overall utility. To address the unmet need for improved therapeutic options, we developed an AAV-based gene therapy to deliver human codon optimized CPS1 (hcoCPS1). AAVs have seen high levels of success in both pre-clinical and clinical studies for other disorders (e.g. Hemophilia B, Spinal Muscular Atrophy) due to their high titers and low immunogenicity; their major limitation is a genome size of 4.7kb. The CPS1 cDNA alone accounts for 4.5kb, making a traditional AAV approach challenging. Despite this, we developed an oversize AAV (oAAV) that combines hcoCPS1 with minimal, liver-specific promoters to determine their therapeutic potential. oAAVs were successfully produced but at reduced titers, leading to dose limits that hindered effectiveness. Mice treated with high dose oAAV typically survived longer than untreated controls, though their chronic ammonia levels were still elevated and their capacity to metabolize acute ammonia loads were limited. To overcome the packaging limitation, we developed a split AAV (sAAV) approach to express hcoCPS1 via the strong CAG promoter, splitting the transgenic cassette in half and subsequently encapsulating into two distinct complementary virions. High dose sAAV-treated mice demonstrated robust lifespan extension and more controlled chronic ammonia and glutamine levels compared to untreated and oAAV-treated mice. Ureagenesis was also restored in female sAAV-treated mice, though males did not demonstrate as robust a rescue in this capacity. Both oAAV and sAAV strategies are amenable to further optimizations to reduce viral load and improve ureagenic output, including alternate regulatory/non-coding cis elements such as alternative minimal promoters, enhancers, and elements to avoid silencing along with enhanced allosteric activation. These strategies represent a fundamental shift towards new therapies for treating CPS1 deficiency patients who have suffered from a paucity of new available treatment options.

CUL3 SUBSTRATE ADAPTOR SPOP REGULATES THE NUCLEAR PORE PROTEIN NUPJ

JOSEPH ONG (Cheml & Biochem/ BMSB, Torres lab)

Cell processes like growth and division are tightly regulated. One such mechanism of regulation is ubiquitination. Ubiquitination can change a protein"s localization or activity, or it can mark the protein for degradation by the ubiquitin proteasome system. The final step of ubiquitination, transferring ubiquitin to the target protein, is mediated by E3 ligases and their substrate adaptors, proteins that allow E3 ligases to be selective in choosing their targets. Understanding the targets of E3 ligases and substrate adaptors, then, is crucial to understanding cell regulation and disease mechanisms linked to misregulation of protein levels and activity. SPOP is a Cul3 E3 ligase substrate adaptor whose targets, such as c-Myc, PD-L1, and ERG, are crucial for cell cycle progression and cancer proliferation. Through a mass spectrometry screen, we identified SPOP as a potential regulator of NupJ, a nuclear pore protein. Knockdown of SPOP via siRNA in HeLa cells leads to increased protein levels of NupJ via immunoblotting, and SPOP and NupJ both co-localize at the nuclear envelope via immunofluorescence microscopy. Moreover, co-immunoprecipitation assays demonstrate that SPOP and NupJ bind to each other in vitro. Similar to overexpression of NupJ, siRNA against SPOP leads to an increase in the number of nuclear envelope defects. Overexpressed NupJ leads to defects in cell division. Our results suggest that SPOP targets NupJ for ubiquitin-mediated proteasomal degradation.

FROM CHEMOPROTEOMIC-DETECTED AMINO ACIDS TO GENOMIC COORDINATES: INSIGHTS INTO PRECISE MULTI-OMIC DATA INTEGRATION

MARIA PALAFOX (Human Genetics, Backus lab)

The integration of proteomic, transcriptomic, and genetic-variant annotation data will improve our understanding of genotype-phenotype associations. Due, in part, to challenges associated with accurate inter-database mapping, such multi-omic studies have not extended to chemoproteomics, a method that measures the intrinsic reactivity and potential "druggability" of nucleophilic amino acid side chains. Here, we evaluated two mapping approaches to match chemoproteomic-detected cysteine and lysine residues with their genetic coordinates. Our analysis reveals that databases update cycles and reliance on stable identifiers can lead to pervasive misidentification of labeled residues. Enabled by this examination of mapping strategies, we then integrated our chemoproteomic data with in silico generated predictions of genetic variant pathogenicity, which revealed that codons of highly reactive cysteines are enriched for genetic variants that are predicted to be more deleterious. Our study provides a roadmap for more precise inter-database comparisons and points to untapped opportunities to improve the predictive power of pathogenicity scores and to advance prioritization of putative druggable sites through integration of predictions of pathogenicity with chemoproteomic datasets.

PROXIMITY BIOTINYLATION REVEALS NOVEL SECRETED DENSE GRANULE PROTEINS OF TOXOPLASMA GONDII BRADYZOITES

AMARA THIND (MBIDP/ IMMP, Bradley lab)

Toxoplasma gondii is an obligate intracellular pathogen that causes serious disease in immunocompromised patients and congenitally infected neonates. During the acute infection, *T. gondii* tachyzoites invade their host cells where they form a parasitophorous vacuole (PV) that is necessary for intracellular survival. During the chronic infection, the parasites switch to slow-growing bradyzoites, and a cyst wall forms around the PV allowing the parasite to persist for the life of the host. To maintain its intracellular niche, *T. gondii* secretes dense granule proteins (GRAs) into the PV space and cyst. Few bradyzoite GRAs have been discovered, and their roles in establishing and maintaining the chronic infection are largely unknown. To identify bradyzoite GRA candidates, we implemented the BioID approach on bradyzoites using biotin ligase BirA* fused to MAG1, a bradyzoite-upregulated GRA. Using this approach, we identified eight new GRAs that were confirmed by endogenous gene tagging and immunofluorescence. Two of these proteins were barely detectable in tachyzoites but readily visualized in the vacuole when the parasites were switched to bradyzoites. We functionally characterized several of these novel GRAs by gene deletion. Disruption of one of these proteins demonstrated that they play important roles in parasite replication in vitro, and its absence results in lower brain cyst burden in vivo. Disruption of another GRA produced normally replicating tachyzoites but resulted in fewer cysts in vivo. Finally, disruption of a third GRA resulted in a dramatic increase in cyst burden and appears to be involved in modulating the host immune response.

REGULATION OF THE CHROMATIN REMODELER SNF2 IN *S. CEREVISIAE*

LAUREN THURLOW (MBIDP/ BBSB, Johnson lab)

Eukaryotic organisms have evolved complex gene regulatory networks to launch coordinated responses to external conditions and stimuli. Under environmental stress, such as nutrient starvation, these responses involve reallocation of cellular resources to stress-induced genes. In addition to transcriptional control, this occurs through several mechanisms including repression of ribosome biogenesis factors, altered translation initiation, and RNA features which modulate translation efficiency and transcript stability. Upon nutrient starvation in *Saccharomyces cerevisiae*, diploid cells undergo meiosis (sporulation in yeast). We have previously shown that the catalytic component of the SWI/SNF chromatin remodeling complex, Snf2, is responsible for shifting gene expression away from intron-rich ribosomal protein genes (RPGs) to enhance splicing of meiotic intron-containing genes (ICGs) during sporulation. Although critical to its activity, the mechanism by which Snf2 protein levels change remains unknown. Here we describe temporally-regulated alternate transcription start sites (TSS) under batch growth and sporulation conditions, which we hypothesize produce SNF2 transcripts with different 5' leaders affecting downstream translation propensity. Specifically, a long isoform of SNF2 contains three upstream open reading frames (uORFs), which we hypothesize control translation of the downstream ORF. I used 5' RACE to identify the previously unannotated TSS of the long isoform, and preliminary RNA analysis suggests that the relative levels of each transcript are dynamic. Protein analysis via Western blotting shows that this regulation indeed affects Snf2 expression. The highly-conserved transcriptional regulator Cbf1 (USF1 in

mammals) appears to play a role in carrying out the down-regulation of Snf2 protein levels, a clue that will be further examined. In light of the conservation of Snf2, investigating its regulation in response to environmental changes in *S. cerevisiae* may carry important implications for Snf2 chromatin remodeling activity in higher eukaryotes.

IMPACT OF MTDNA MUTATIONS AND TRANSFER ON TUMOR GROWTH DYNAMICS

AMY YU (MBIDP/ CDB, Teitell lab)

Mitochondrial dysfunction and mutations in mitochondrial DNA (mtDNA) are common features of many cancers, frequently associated with increased tumor growth and invasiveness. Although it is known that mitochondrial ATP and metabolites influence many aspects of cellular metabolism, survival, and proliferation, it remains unclear how differences in mtDNA sequence impact tumorigenesis and metastasis. Critically, it is unknown whether mtDNA mutations are sufficient to alter the growth kinetics of a tumor, which represents a fundamental lack of understanding with potential clinical implications. To address this, we utilized an in vitro method developed in our lab to transfer whole mitochondria from one cell type to another (MitoPunch), effectively engineering the mtDNA sequence. We generated tumor cells with known mtDNA mutations in isogenic nuclear backgrounds of B16 melanoma and 4T1 mammary carcinoma cells. By comparing the growth dynamics, metabolism, and invasiveness of these cells in both in vitro and in vivo conditions, we will gain clear insights into how mtDNA affects cancer growth and metastasis independently of the nuclear genome. Furthermore, we will assess the cell's ability to modulate its mtDNA content over time in vivo, which has been shown to occur in conditions of severe mitochondrial dysfunction. Understanding the dependence of cancer cells on their mtDNA will provide fresh insight into tumor heterogeneity and evolution in cancer metabolism as a whole.

ELUCIDATING THE EFFECT OF GUT BACTERIAL CARBOHYDRATE UTILIZATION ON HOST DIETARY PREFERENCE

KRISTIE YU (MBIDP/ IMMP, Hsiao lab)

Obesity is a rapidly growing global epidemic. Since one contributing factor is the availability and consumption of high-calorie foods, interventions to alter preferences towards healthier foods may prevent obesity. This requires an understanding of the biological determinants of food preference. The gut microbiota regulates host metabolism by interacting with dietary nutrients, particularly non-digestible complex carbohydrates, and liberating metabolites to the host, such as short chain fatty acids (SCFAs) which can reduce host appetite. Whether microbial fermentation impacts host food choice in mammals remains unknown. We hypothesize that nutrient preferences of select gut bacteria will condition host dietary preferences, via bacterial metabolites signaling to neuronal circuits underlying homeostatic feeding. *Bacteroides* species, which comprise ~25% of the human gut microbiota, digest fibers via genetically-encoded polysaccharide utilization loci (PUL). *Bacteroides thetaiotaomicron* (theta) and *B. ovatus* express PUL variants that restrict their metabolism to fructans with different linkages: *B. theta* utilizes levan with β2-6 linkages, whereas *B. ovatus* utilizes inulin with β2-1 linkages. Here we show that colonization of germ-free mice with *B. theta* or *B. ovatus* modifies host preference for a levan- or inulin-restricted diet. Mice colonized with *B. ovatus* develop a preference for levan diet in a food choice task after exposure to both diets for 28 days, while those with *B. theta* do not show preference for either diet. A mechanistic understanding of how gut microbes influence feeding behavior will raise the potential of using microbial manipulation to ameliorate obesity.

DESIGN OF 3D PROTEIN CRYSTALS

KYLE MEADOR (Chem & Biochem/ BMSB, Yeates lab)

Developments over the past 20 years have enabled the accurate design of proteins to serve all manners of form and function. Our lab has been at the forefront of applying these ideas towards large symmetric protein assemblies, increasing the size and complexity with which biotechnologies are built. Despite much progress, the development of infinitely repeating materials, best characterized by 3D crystals, has lagged behind finite materials such as cubic or icosahedral capsid assemblies. Our group has laid out shared principles and geometric rules that should apply equally to the construction of finite and infinitely repeating materials; the essence is a requirement for rigidly orienting two oligomeric protein components with respect to one another. So far this challenge has not been demonstrated for three-dimensionally repeating protein materials (i.e. protein crystals) made out of two components. Our recent work has demonstrated conceptual advances towards creating such materials, including simultaneously constraining mathematical, biochemical, and evolutionary observations to satisfy design criteria. We have applied these concepts into a comprehensive design framework and have tested this methodology on 48 promising crystal candidates. Ongoing work to verify our design hypothesis experimentally will inform on our novel techniques. With success, we will inform on the best practices for designing repeating protein assemblies enabling simple design of a vast number of materials with diverse applications in biotechnology and nanomedicine. Simultaneously, our novel design methodologies will provide rich experimental evidence regarding protein-protein interface design. Finally, success of our work allows an easy platform to study crystal formation mechanisms with reduced parameter space. In conclusion, this work seeks to demonstrate that design of atomically precise, yet infinitely ordered materials is possible and will be the cornerstone of the next generation of biotechnological advances.

POSTER SESSION 2
3:00 - 4:00 PM

HETEROGENEITY AND RELATED INTERACTIONS WITH EQTL IN YEAST

NOAH ALEXANDER (MBIDP/ GREAT, Kruglyak lab)

An advantage of model organism-based studies of complex trait variation is that large populations of progeny can be generated and characterized. The applications of these family-based linkage studies to genome-wide phenotypes in yeast using high-throughput assays have been instrumental in developing an understanding of the genetic basis of molecular trait variation. The development of single cell RNA sequencing affords an opportunity to extend the study of complex trait variation to a much higher number of individuals by replacing individual colonies and bulk RNA seq (or another assay) with single cell phenotypes generated from library preparations of yeast segregants. Additionally, single cell RNAseq data afford characterization of heterogeneity within cultures such that nuanced statements can be made about the relationship between the sources of heterogeneity and their interactions with QTL. To this end, we have generated single cell RNA seq data for haploid parents, diploid parents, and segregants of a cross between strains BY and RM. First, we recapitulated prior eQTL results from bulk RNA seq using scRNA data. Next we used the haploid parent data to develop penalized logistic regression classifiers of cell cycle phase (evaluated in diploid and haploid segregants) along with other annotations of the sources of heterogeneity within the datasets. We then used these annotations to look for interactions with eQTL.

ASSESSING THE EXISTENCE OF NERIN1 ISOFORMS IN THE DEVELOPING SPINAL CORD

SANDY ALVAREZ (MBIDP/ CDB, Butler lab)

The function of the nervous system is dependent on the correct formation of neural circuits during development. Circuits are generated when growth cones at the tips of axons use molecular cues in the environment to guide axon extension. Netrin1 is an axon guidance cue first thought to acts as a long-range, diffusible, chemoattractant that emanates from the floor plate (FP). However, recent work by the Butler lab and other groups, has suggested this model is incorrect. In the mouse spinal cord, netrin1 is expressed by both FP cells and neural progenitor cells (NPCs) in the ventricular zone (VZ). In the absence of either netrin1 or its receptor Dcc, axons innervate the VZ and commissural axons either stall or defasciculate. These phenotypes are only observed when netrin1 is removed from NPCs and not the FP cells, suggesting NPC-derived netrin1 is responsible for guiding axon extension. Our studies suggest that NPC-derived netrin1 is deposited on the pial surface (margin) of the spinal cord, where it acts as an adhesive substrate that promotes commissural axon outgrowth. My objective is to define the mechanisms that allow netrin1 to be transported to the pial surface of the spinal cord. In the visual system, netrin1 can be cleaved into fragments, isoforms, with unique spatial and biological properties. This led me to believe that cleavage of netrin1 facilitates its transport to the pial surface. My preliminary data suggests that netrin1 isoforms exist in the spinal cord, and that netrin1 is differentially cleaved to permit its localization to different regions of the spinal cord. Furthermore, my data suggests that netrin1 transport is mediated by the motor protein Kif1a. Investigating these mechanisms is critical to 1) gaining a better understanding of the basis of neurodevelopmental disorders and 2) the repair and regeneration of damaged circuits.

DETERMINING SOURCES AND TARGET CELLS OF SPP1 IN DYSTROPHIC MUSCLE NICHE USING SINGLE-CELL RNASEQ

RAQUEL ARAGON (MBIDP/ CDB, Spencer lab)

In DMD, chronic cycles of degeneration and regeneration lead to aberrant intramuscular inflammation and accumulation of fibrosis. Our previous work showed that Spp1 exacerbates progression of muscular dystrophy. We showed that Spp1 promotes muscle fibrosis and shifts macrophages to a pro-inflammatory phenotype. However, the cellular source of Spp1 and its target cells in dystrophic muscle are unknown. It is likely that different cellular sources of Spp1 could have disparate effects on target cells. In this study, Spp1 floxed mice were crossed to muscle stem cell (MuSC)-specific and myeloid-specific Cre drivers to parse how Spp1 derived from these cell types promotes disease, and to dissect the impact of local sources of Spp1 on the dystrophic cellular milieu. To dissect the source of Spp1, we conducted functional testing and assessed how Spp1 ablation affected muscle performance. To characterize the effect of cell specific sources of Spp1 on cellular targets, we carried out scRNAseq. Functional testing showed a mild improvement of MuSC-specific (Pax7-Cre) Spp1 conditional knockout. Bulk RNAseq of sorted Pax7(+) cells revealed Spp1 ablation had an autocrine effect on Pax7 cells including induction of pro-myogenic genes and inhibition of inflammatory and fibrotic genes. scRNAseq analysis revealed that Pax7 cKO shifted macrophage phenotypes. Myeloid-specific (Lyz2) Spp1 cKO showed a significant improvement in muscle function. scRNAseq revealed that macrophage-specific Spp1 cKO led to ablation of a specific population of Pdgfra(+) stromal cells with upregulated ApoD expression. These findings suggest that different cellular sources of Spp1 exert diverse downstream effects on cells in dystrophic muscles.

EPIGENETIC MODIFICATION IN NK CELL ANTI-TUMOR RESPONSES

MANDY CHENG (MBIDP/ IMMP, Su lab)

Natural Killer (NK) cells play a crucial role in cancer immunosurveillance by expressing multiple cell surface receptors that recognize stress-induced ligands on malignant cells. Engagement of NK cell surface receptors elicit an intricate signaling network that results in the release of cytotoxic granules and immunomodulatory cytokine production. Low frequency of NK cells in human peripheral blood correlates with increased cancer incidence, indicating importance of NK cells in tumor immunity. However, the factors that regulate NK cell maturation remain unclear. Accumulating evidence suggests a critical role for epigenetic regulation in immune cell differentiation. Interestingly, our preliminary data implicate an epigenetic regulator, Ubiquitously Transcribed Tetratricopeptide repeat on chromosome X (UTX), in control of NK cell maturation and responses. Our preliminary data demonstrates NK cell-specific deletion of UTX results in abnormal proportions of NK cell maturation subsets suggesting that UTX controls NK cell maturation. Interestingly, a recent study shows UTX demethylase activity is

oxygen-dependent. Moreover, aberrant growth and lack of blood supply causes many tumor microenvironments (TME) to be hypoxic. Therefore, maturation defects in response to UTX-deficiency in NK cells may be linked to the inhibition of UTX activity in a hypoxic TME. Since NK cells are the first line of defense against a multitude of cancers, much interest surrounds the potential use of CAR-transduced NK cells for cancer immunotherapy. Therefore, understanding epigenetic modulation of NK cells in the context of hypoxic TMEs are critical to enhance the efficacy of both endogenous NK cell responses and potential NK cell cancer therapies.

TRIM28 IS ESSENTIAL FOR MOUSE GERMLINE DEVELOPMENT

JONATHAN DIRUSSO (MBIDP/ CDB, Clark lab)

The germline is responsible for transmitting the genome between parent and offspring, therefore the maintenance of germline integrity is critical for offspring health. During the initial stages of mammalian germline development retrotransposons are repressed despite global loss of DNA methylation from the germline. While most retrotransposons in the mammalian genome have degraded and are no longer capable of active transposition, some young retrotransposons retain the ability to mobilize, presenting a potential threat to germline genome integrity and, consequently, fertility if abnormally expressed. Here we assess the role of tripartite-motif containing-28 (Trim28) in maintaining repression of young transposition competent retrotransposons during global loss of DNA methylation in mouse primordial germ cells (PGCs). Trim28 binds sequence-specific KRAB-zinc finger proteins and recruits epigenetic modifiers such as the methyltransferase Setdb1 to establish repressive marks. Using a conditional-knockout approach in PGCs, we show that loss of Trim28 leads a derepression of the young IAPEz family of retrotransposons, similar to previously reported IAP derepression in Trim28 KO mouse Embryonic stem cells. We also observed a significant reduction in PGC number in both male and female embryos by E13.5, roughly coincident with gonad dimorphism. Unlike loss of some epigenetic modifiers in PGCs, RNA-Seq of the mutant and control PGCs indicates Trim28 loss does not induce a precocious entry into meiosis. These findings demonstrate an essential role for Trim28 in germline development, although the exact mechanism by which TRIM28 protects against PGC loss prior to E13.5 remains enigmatic.

TOWARDS A MECHANISTIC UNDERSTANDING OF A TYPE III POLYKETIDE SYNTHASE INVOLVED IN CANNABINOID BIOSYNTHESIS

KRISTOFER GONZALEZ-DEWHITT (MBIDP/ BBSB, Abramson lab)

Plant-specific type III polyketide synthases catalyze the iterative condensation of coenzyme-A thioesters to produce a variety of polyketide scaffolds. In Cannabis sativa, a polyketide synthase generates a linear poly-β-keto tetraketide, which downstream enzymes cyclize to form tetrahydrocannabinol or cannabidiol. Crystal structures of this polyketide synthase reveal the protein's catalytic cysteine undergoes irreversible oxidation to yield an inactive enzyme, making biochemical and biophysical characterization impossible. Current efforts are focused on identifying and preventing this inhibitory oxidation reaction in order to retain an active enzyme for characterization. The overarching goal of the project is to develop a biosynthetic approach that improves cannabinoid production for pharmaceutical purposes.

SYNTHESIS OF FLUORINE-18 LABELED SMALL MOLECULES FOR USE IN PET IMAGING

BALDWIN LIWANAG (Chem & Biochem, Murphy lab)

Bioorthogonal reactions are versatile in function; current and potential applications of these reactions include in situ drug delivery, uncaging reactions, and bioimaging. Highly selective bioorthogonal reactions have been used to image relevant biochemical processes, such as cancer metabolism, disease progression, and biomolecule localization. One area of research we are interested in exploring is immune cell imaging; imaging of immune cells allows for non-invasive visualization of immune cell response and its localization. Our research goal is to develop new bioorthogonal reactions with favorable kinetics for use in positive emission tomography (PET) and near-infrared fluorescent (NIRF) imaging. Our group's primary focus is the synthesis of Fluorine-18 labeled compounds for PET imaging.

EED REGULATES MOUSE PRIMORDIAL GERM CELL DIFFERENTIATION

MATTHEW LOWE (MBIDP/ CDB, Clark lab)

Primordial Germ Cells (PGCs) are the source of the entire adult germline and their proper differentiation is essential for the maintenance of fertility. PGCs are specified early in the embryo and undergo extensive epigenetic reprogramming before differentiation. Two major epigenetic reprogramming events occur during PGC development, including the genome wide depletion of DNA methylation (5mC) and enrichment of histone 3 lysine 27 trimethylation (H3K27me3). While the loss of 5mC is one of the most well characterized epigenetic changes in PGCs, the concurrent enrichment of H3K27me3 is considerably less studied. Through the use of a germ cell specific EED conditional knockout mouse (ECKO), we show that while EED and H3K27me3 are not necessary for the global depletion of 5mC, they are necessary to prevent precocious PGC differentiation. These findings are consistent with that of other epigenetic knockouts, particularly the conditional depletion of DNMT1 which maintains DNA methylation at a small number of germ cell differentiation genes in the absence of UHRF1. To evaluate the relationship between H3K27me3 and 5mC, we identified the late demethylating promoters in PGCs, and show that these promoters are also enriched in H3K27me3. Additionally, through co-IP we identified an interaction between EED and DNMT1. In summary, our findings suggest the developmental timing of PGC differentiation is regulated by a unique interplay between EED and DNMT1 which occurs in the absence of UHRF1.

SILYL TOSYLATES AS PRECURSORS TO CYCLOHEXYNE, 1,2-CYCLOHEXADIENE, AND 1,2-CYCLOHEPTADIENE

MATTHEW MCVEIGH (Chem & Biochem, Garg lab)

Transient strained cyclic organic molecules, such as strained cyclic alkynes and allenes, are valuable building blocks in the synthesis of bioactive natural products and drug candidates. Although these strained intermediates are typically accessed in situ from the corresponding silyl triflate, the instability of some silyl triflates warrants the development of alternative precursors. We present the syntheses of silyl tosylate precursors to cyclohexyne, 1,2-cyclohexadiene, and 1,2-cycloheptadiene. The resultant strained intermediates undergo efficient trapping in situ to give diverse products of potential value to pharmaceutical chemists.

ENGINEERING A YEAST-BASED PLATFORM FOR PRODUCTION OF NOVEL MONOTERPENE INDOLE ALKALOIDS

JOSHUA MISA (Chem & Biomolecular Engineering, Tang lab)

Monoterpene indole alkaloids (MIAs) are an expansive class of plant natural products, many of which have been named on the World Health Organization's List of Essential Medicines. Their biological activities include anti-cancer, anti-malarial, anti-addiction, and more. However, MIAs are some of the costliest small-molecule drugs due to low production from native hosts. Thus, a more sustainable and reliable source of these drugs is critical to meet global demand. The model eukaryote, *Saccharomyces cerevisiae* (Baker's yeast), has proven to be an effective host for production of numerous plant natural products in recent years. Here we report the development of a yeast-based platform for high-titer production of the universal MIA precursor, strictosidine, using a combination of synthetic biology and metabolic engineering techniques. Our fed-batch platform produces 5 mg/L strictosidine starting from the inexpensive commodity chemical, geraniol, and is the highest titer reported thus far. Additionally, our robust platform can produce strictosidine analogues through feeding modified substrates to our host. Our platform will enable future reconstitution of downstream biosynthetic pathways towards production of more elaborate MIAs such as the frontline cancer therapeutic vinblastine, the anti-addiction agent ibogaine, and their novel analogues.

ELUCIDATING THE ROLE OF EPIGENETIC REGULATORY PROTEINS IN INTELLECTUAL DISABILITY SYNDROMES

ABRIL MORALES (MBIDP/ CDB, Lowry lab)

Many severe intellectual disability (ID) syndromes due to genetic mutations are associated with epigenetic regulatory genes. However it remain unclear why many ID syndromes are caused by loss of function of epigenetic regulatory proteins. Research in Rett syndrome has failed to determine exactly why loss of Methyl-CpG Binding Protein 2 (MeCP2) leads to defects in dendritic branching, and neurophysiological defects in humans. Thus, it is imperative to comprehensively study how MeCP2 fundamentally works in the cell in order to understand how loss of this protein leads to senescence. While typically described as a DNA binding protein, early studies of MeCP2 described RNA binding activity I hypothesize that RNA binding and processing is an alternative mechanism for MeCP2 that underlies the neurophysiological defects due to loss of this protein. Fibroblasts from patients with these intellectual disabilities were programmed into induced pluripotent stem cells (iPSCs). Neuronal cell lines were then derived from these iPSCs to produce in vitro models of the disease. To characterize the possible RNA processing functions of MeCP2 in the nucleus, I determined the localization of MeCP2 with markers of subnuclear domains. I found MeCP2 colocalizing to SC35, indicating a possible role for MeCP2 in pre-mRNA splicing mechanisms within nuclear speckles. I also found larger nucleoli in mutant MeCP2 neuronal cell lines compared to control indicating stress.

DEWAR HETEROCYCLES AS VERSATILE INTERMEDIATES IN SYNTHESIS

SEPAND NISTANAKI (Chem & Biochem, Nelson lab)

Aromatic molecules make up a vast portion of our feedstock chemicals given their wide abundance in nature. However, due to their inherent stability imparted by these molecules' resonance energy, their reactivity is often poor. To this end, dearomative reactions have attracted the attention of the synthetic chemistry community as a powerful strategy for accessing complex three-dimensional functionality from readily available "flat" compounds that are typically thought to be too inert to undergo productive chemical reactions. Aromatic heterocycles are known to engage in valence isomerization reactions under photochemical conditions to generate their dearomatized Dewar isomers. This valence isomerization effectively unveils new reactive functional group handles, providing an attractive platform for generating densely functionalized 3D building blocks for organic synthesis. We have demonstrated the strategic use of these intermediates (namely Dewar pyrones, Dewar pyridones, and Dewar pyridines) in a variety of synthetic applications. As one such example, we have been able to access the bioactive natural product Vibralactone, which is an inhibitor of pancreatic lipase amongst other notable bioactive activities. Utilization of a Dewar pyrone intermediate allowed for a concise 4-step sequence to access the natural product, which is the shortest synthetic route reported to date. Furthermore, this platform allows for extensive SAR by rapid analog development. Additionally, we have demonstrated the use of Dewar heterocycles as versatile monomers for ring-opening metathesis polymerization (ROMP), providing access to a variety of unique polymers with potential applications in biomedical/ biomimetic materials and conducting materials.

MECHANISM AND FUNCTION OF MEMBRANE HOMEOSTASIS OF SORTASE MODULATED BY AN EVOLUTIONARILY CONSERVED PROTEIN

NICHOLAS RAMIREZ (MBIDP/ IMMP, Ton-That lab)

Pili are fiber-like appendages present on the surface of both Gram-negative and Gram-positive bacteria and play key roles in adherence, motility, and virulence. Unique to Gram-positive bacteria are the covalently-linked pili that are assembled and anchored to the cell wall via the cysteine

transpeptidase enzymes called sortases. In the model organism *Actinomyces oris*, pilus assembly occurs by a biphasic mechanism, in which pilus polymerization catalyzed by a pilus-specific sortase is followed by cell wall anchoring modulated by the housekeeping sortase SrtA. Previous studies demonstrate the role of SrtA in regulating pilus length since a mutant devoid of srtA produces extremely long pili that fail to mediate polymicrobial interactions. How this membrane-bound sortase enzyme controls pilus length is not well understood. We show here that a small membrane protein, termed SafA, encoded by the safA gene located immediately downstream of srtA, is evolutionarily conserved among Gram-positive Actinobacteria, but absent in Firmicutes. Remarkably, deletion of safA resulted in proteolytic cleavage of SrtA and secretion of its cleaved polypeptide into the extracellular milieu. Like the srtA mutant, the safA mutant exhibited altered cell morphology, production of exceedingly long pili, and inability to interact with *Streptococcus oralis*. Significantly, ectopic expression of srtA from *A. oris*, *Corynebacterium diphtheriae*, and *Corynebacterium matruchotii* in the srtA mutant rescued the aforementioned defects in *A. oris*. These results support the hypothesis that pilus extension is modulated by membrane homeostasis of SrtA via the membrane-bound protein SafA and that the SafA-mediated mechanism is conserved in Gram-positive Actinobacteria.

DETERMINING WHETHER RNAI CORE COMPONENTS ARE INVOLVED IN MAMMALIAN PERICENTROMERIC REGULATION

RAFAEL SANDOVAL (MBIDP/ BBSB, Zamudio lab)

RNA interference (RNAi) is a highly conserved gene regulatory process that utilizes small RNA to target messenger RNA (mRNA) for transcriptional repression. Although the role RNAi plays in posttranscriptional regulation has been well-characterized in a myriad of eukaryotic systems, studies show RNAi factors Argonaute (Ago) and Dicer also serve essential roles in establishing and maintaining the heterochromatin state of the pericentromere therefore regulating its transcriptional output and higher order structure. Although the intersection between RNAi and heterochromatin assembly is well resolved in most eukaryotic systems, it remains elusive in mammals. To characterize the Ago-mediated regulation at the mammalian pericentromere, we utilize a mouse embryonic stem cell (mESC) system that has an Ago1-4 null background and a doxycycline inducible Ago2. Here, we confirm the existence of multiple pericentromeric ncRNA species that vary in size. Furthermore, with the loss of Ago proteins, we observe a change in the size heterogeneity and an increased abundance of pericentromeric ncRNAs. Using live-cell imaging and histone H2B-GFP cell lines, we observe colcemid treated Ago null cells bypass the mitotic spindle checkpoint at higher frequency compared to cells expressing Ago2.

HALTING AND VISUALIZING HEME ACQUISITION BY PATHOGENIC *STAPHYLOCOCCUS AUREUS* USING A PHOTOCAGED HB RECEPTOR

JESS SOULE (Chem & Biochem, Rodriguez & Clubb labs)

The Center for Disease Control has identified *methicillin resistant Staphylococcus aureus* (MRSA) as a serious threat, which annually in the United States is responsible for over 300,000 hospitalizations and 10,000 deaths. *S. aureus* acquires the essential nutrient iron from human hemoglobin (Hb), a process required for virulence and a potential target for new therapeutics. Iron scavenging from Hb is mediated by nine iron-regulated surface determinant proteins (IsdA-I). In the first step of this pathway, the closely related peptidoglycan-anchored IsdH and IsdB receptors bind Hb and extract its heme cofactors. IsdH accomplishes heme extraction via a tridomain unit consisting of its second and third NEAr iron Transporter (NEAT) domains and a helical linker. A structure of the IsdH:Hb complex has provided insight into how the receptor distorts Hb to promote heme release, and has suggested that heme flows through a channel to the third NEAT domain. Additional structures of IsdH bound to heme analogs have revealed a binding pocket in which Y642 axially coordinates the heme iron. Time-resolved crystallography will be used to define the molecular basis of heme transfer by creating a photo-regulated receptor. 2-nitropiperonyl tyrosine (NPY) cleaves to produce tyrosine on irradiation with UV light and has been incorporated into IsdH in place of Y642 as a means of achieving photo-regulation. Here we present initial work toward incorporating the unnatural amino acid and verifying IsdH binding pocket occlusion.

IN SILICO ENERGETIC ANALYSIS OF THE AMYLOID FOLD

SAMANTHA ZINK (Chem & Biochem/ BMSB, Rodriguez lab)

Protein folding is considered a global optimization problem, where the protein works towards optimizing structural features such as backbone and sidechain angles while burying hydrophobic residues away from the solvent. Success in the computational simulation of this process has been demonstrated for both globular and membrane proteins as they typically have one native conformation present at a global free energy minimum. However, amyloid proteins are known to exhibit structural polymorphism which is represented by many local minima near the global free energy minimum, making in silico modeling and prediction difficult. This challenge hinders our important goal of understanding the amyloid fold, which is implicated in numerous diseases including Alzheimer's and Parkinson's. To better understand the energetics of the amyloid fold, I am building a computational pipeline that evaluates a pool of experimentally determined amyloid structures. In brief, I score the amyloid with an energy function, break that score down on a per-residue basis, allowing me to identify stabilizing and destabilizing features. This type of analysis will deepen our understanding of how polymorphs are related to each other on an energetic level and opens up future applications in amyloid design and de novo structure prediction.